Model of multisite tau protein phosphorylation for prediction of sensitivity to therapeutic interventions.

Alexander Stepanov, Tatiana Karelina, Nikolay Markevich, Timothy Nicholas

Institute for Systems Biology, Moscow, Russia, stepanov@insysbio.ru

Pfizer Global R&D, Groton, CT, USA, timothy.nicholas@pfizer.com

Introduction. Microtubule-associated protein tau (MAPT) stabilizes microtubules regulating cell architecture and cargo transport. As an intrinsically disordered protein (IDP) in unbound state tau accepts an ensemble of conformations more or less vulnerable to modification such as phosphorylation. More than 80 residues of tau can be potentially phosphorylated by a number of kinases. Hyperphosphorylation of tau is associated with tau aggregation and neurodegeneration. Development of the model of tau phosphorylation would be helpful for searching the targets for pharmacological interventions and candidates for biomarkers of pathology progression.

Model development. Several mathematical models describing a variety of molecular mechanisms of the multisite phosphorylation have been developed [1]. A protein with N phosphorylation sites can exist in 2^N phosphorylation states. The reduction of such high-dimensional phosphorylation state spaces to a smaller number of functional states may occur on two levels. First, for some tau sites sequential mechanism is applied. For random mechanism, the reduction of state spaces is achieved through assumptions about independent phosphorylation events [2].

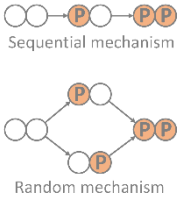

Fig.1. Mechanisms of multisite protein phosphorylation towards to the independence of phosphorylation events. GSK3beta phosphorylates tau by random mechanism except 404 and 396 sites, which are phosphorylated by sequential mechanism. CDK5 phosphorylate all sites by random mechanism. Phosphatase PP2A dephosphorylates tau protein by random mechanism.

Our model is based on the following assumptions:

1) Residues are phosphorylated independently from each other but compete with each other for binding with kinase. Phosphorylation could influence subsequent phosphorylation of other sites (positive and negative priming), this influence is taken into account by parameter (alpha), describing conformational changes.

2) Residues could be phosphorylated only partially, which was confirmed by *in vitro* studies [3] and by incorporation of total phosphate into tau [4].

3) Michaelis constants for kinases for all residues are equal to each other.

Each residue can be in opened or closed state. Each residue in open state is considered as substrate, and its phosphorylation follows standard Michaelis-Menten kinetics with substrate competition (assumption 1) and K_M equal for all residues (assumption 3):

$$r_i \rightarrow P_i$$

$$\frac{dP_i}{dt} = \frac{E_0 \cdot k_{cat}^i \cdot r_i}{K_M + \sum r_i}$$

Partial residue phosphorylation is described through initial state of the system:

$$r_i(0) = alpha_i * tau$$

$$N = \sum alpha_i$$

Here, alpha is a probability of residue to be in open state at the initial moment of the *in vitro* experiment used for calibration, and then N would be stoichiometry of tau phosphorylation when system is close to steady state. This approach helps in description of experiments with one kinase, but has limitation when applied to cases with several kinases. For these cases alpha parameters are taken equal to 1, i.e. for simulations on long times we assumed very slow tau conformational transitions leading to complete opening of the sites. For dephosphorylation description, phosphorylated state of residue was considered as substrate.

Data for model calibration includes kinetics of tau phosphorylation [4] and de-phosphorylation [5] at individual sites and total tau phosphate incorporation [4].

Results and discussion. Model parameters were calibrated against experimental data (see examples on **Fig.2** and **3**) and 95% confidence intervals for them have been calculated.

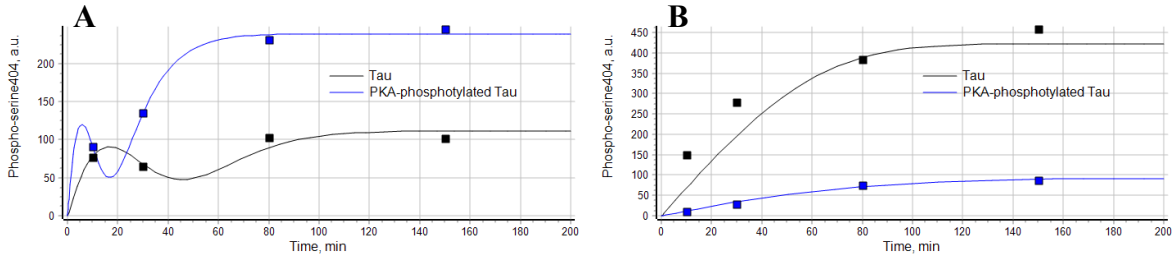

Fig.2. Kinetics of tau phosphorylation at S404 with **A)** GSK3beta. The transient peak is caused by sequential phosphorylation (first S404, then S396) by GSK3beta in contrast to kinetics of tau phosphorylation at S404 with CDK5 **(B)** when S396 and S404 residues are phosphorylated independently by random mechanism.

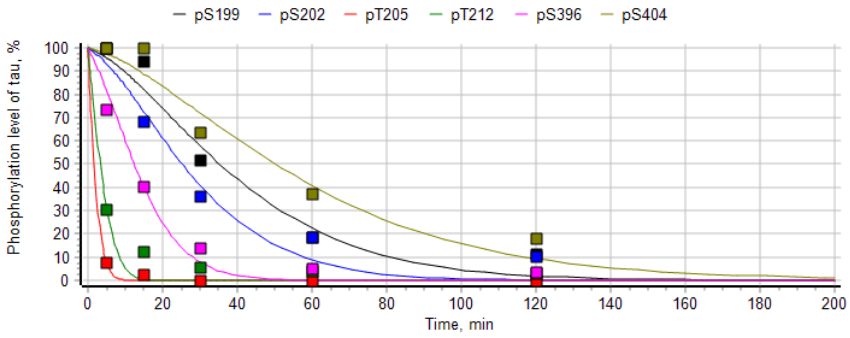

Fig.3. Kinetics of tau de-phosphorylation at individual phosphorylation sites by PP2A.

Pseudoglobal sensitivity analysis was also performed to explore: 1) how the uncertainty in the output of a mathematical model can be apportioned to the different sources of uncertainty in its inputs; 2) how the different tau states are sensitive to potential disturbance of the system. Phosphorylation states of residues and total tau phosphorylation stoichiometry (a) were chosen as endpoints. For selected parameters (inputs, enzyme concentrations and catalytic constants), 10^5 random sets of parameters were generated from the lognormal distribution. For each set of parameters, endpoints were calculated and then for each pair (endpoint-parameter) Pearson's correlation coefficients were calculated and presented on a heatmap.

Kinetic data for p38 kinase (gamma subunit) were not available, and we have chosen parameters so that 205 residue phosphorylation would be dependent strongly on p38gamma activity [6].

Sensitivity analysis (**Fig.4**) revealed that endpoints are more sensitive to kinase and phosphatase level comparing to individual catalytic constants (kcat) of residues. There is no cross-dependence between residues. Tau phosphorylation is most sensitive to PKA and GSK3beta activity in contrast to CDK5 and p38gamma. We can conclude that, for example, for decrease of PHF1 phosphorylated tau (i.e. phosphorylation on residues 396 and 404) GSK3beta would be the best target, while pS202 would be the most sensitive biomarker for this therapy.

It's well known that hyperphosphorylation of tau is associated with tau aggregation. But not all phosphorylation events on tau are associated with toxicity. Increased phosphorylation of tau occurs during fetal development and transiently in hibernating mammals. One phosphorylation pattern leads to destabilizing of microtubules and tau aggregation, but other one may be protective against degeneration.

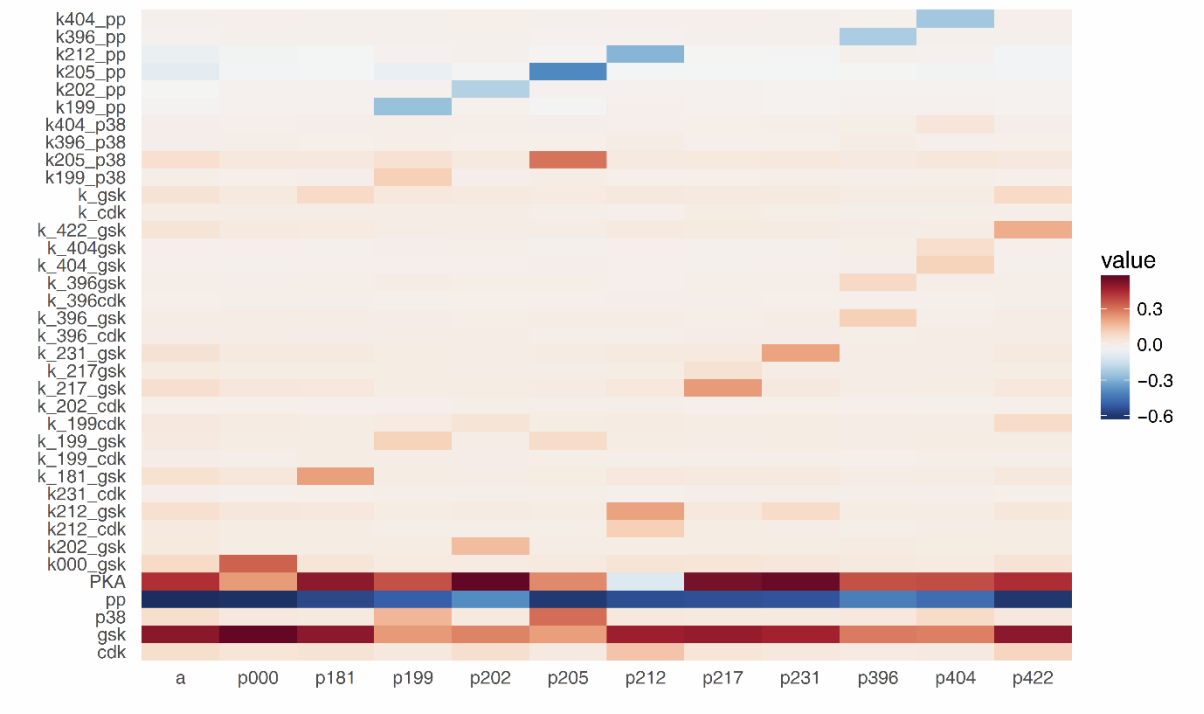

Fig.4. Sensitivity analysis. Heatmap of Pearson's correlation coefficients.

This approach illustrates how analysis of *in vitro* kinetics allows for choice of best candidates for therapeutic targets and biomarkers.

References.

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